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TOWNSEND and TOWNSEND and CREW LLP

By: Malinda Dogt

PATENT
Attorney Docket No.: 020130-000111US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

WANG

Application No.: 09/870,353

Filed: May 30, 2001

For: IMPROVED NUCLEIC ACID MODIFYING ENZYMES

Customer No.: 20350

Confirmation No. 8319

Examiner: Richard Hutson

Technology Center/Art Unit: 1652

REVISED APPEAL BRIEF UNDER 37 C.F.R. § 41.37

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Commissioner for Patents
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Alexandria, VA 22313-1450

Sir:

This revised Appeal Brief is filed in response to the Notification of Non-Compliant Appeal Brief dated October 17, 2008. The Brief is a revision of the Appeal Brief filed September 23, 2008. The revisions correct the status of claims 20 and 33 in the **Status of the Claims** section and removes references to those claims in the **GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL** and **ARGUMENTS** sections.

As indicated on the Evidence Appendix (page 32) accompanying this revised Brief, the Evidence Appendix was submitted with the Appeal Brief filed September 23, 2008. Further to an inquiry to the Patent Appeal Specialist that signed the Notification, it is Appellants' understanding that it is not necessary to re-submit the Evidence Appendix with this revised Brief as it is identical to the previously filed Evidence Appendix.

The fee pursuant to 37 CFR §41.20(b)(2) was paid in the Appeal Brief filed September 23, 2008. Appellants believe no additional fees are due. However, should additional fees be due, please deduct them from deposit account 20-1430.

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I. REAL PARTY IN INTEREST

Bio-Rad Laboratories, Inc. is the assignee of the above-referenced patent application by an assignment from MJ Bioworks, Inc. and thus, the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals, interferences, or judicial proceedings at this time.

III. STATUS OF THE CLAIMS

Claims 1-14, 16, 18-21, 31, and 33 are cancelled.

Claims 15, 17, 22-30, 32, and 34-44 are pending.

No claims are withdrawn from consideration but not cancelled.

No claims are allowed.

No claims are objected to.

Claims 15, 17, 22-30, 32, and 34-44 are rejected.

Claims 15, 17, 22-30, 32, and 34-44 are being appealed.

IV. STATUS OF AMENDMENTS

No amendments after the final Office Action were submitted. Claims 15, 17, 22-30, 32, and 34-44 on appeal herein are as amended in the Response to the Office Action filed February 4, 2008.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A. Claim 15-Independent

The subject matter of independent claim 15 relates to a protein comprising two joined heterologous domains: (1) a sequence non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 75% sequence identity to SEQ ID NO:2; and (2) a DNA polymerase domain; where the presence of the sequence non-specific double-stranded nucleic acid binding domain enhances the processivity of the polymerase domain compared to an identical protein that does not have the sequence non-specific double-

stranded nucleic acid binding domain joined to it. *Support for this claim can be found, e.g., on page 13, line 32 bridging to page 14, line 13.*

B. Claim 30-Independent

The subject matter of independent claim 30 relates to a protein comprising two joined heterologous domains: a sequence non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 75% sequence identity to the Sac7d sequence set forth in amino acids 7-71 of SEQ ID NO:10; and a DNA polymerase domain, where the presence of the sequence non-specific double-stranded nucleic acid binding domain enhances the processivity of the polymerase domain compared to an identical protein that does not have the sequence non-specific double-stranded nucleic acid binding domain joined thereto. *Support can be found, e.g., in SEQ ID NO:10 and at page 12, lines 8-9 and page 14, lines 5-9.*

C. Claim 43-Dependent

The subject matter of dependent claim 43 relates to a protein as in claim 15 where the sequence non-specific double-stranded nucleic acid binding domain comprises an amino acid sequence that has at least 85% sequence identity to SEQ ID NO:2. *Support can be found, e.g., on page 14, lines 5-9.*

D. Claim 44-Dependent

The subject matter of dependent claim 44 relates to the protein of claim 30, where the sequence non-specific double-stranded nucleic acid binding domain comprises an amino acid sequence that has at least 85% sequence identity to the Sac 7d sequence set forth in SEQ ID NO:10. *Support can be found, e.g., in SEQ ID NO:10 and at page 12, lines 8-9 and page 14, lines 5-9.*

E. Claims 34-Dependent

The subject matter of dependent claim 34 relates to the protein of claim 30, where the sequence non-specific double-stranded nucleic acid binding domain comprises an amino acid sequence that has at least 90% sequence identity to the Sac 7d sequence set forth in SEQ ID NO:10. *Support can be found, e.g., in SEQ ID NO:10 and at page 12, lines 8-9 and page 14, lines 5-9.*

F. General Summary of Claimed Subject Matter

The pending claims relate to polymerase proteins that are defined by two domains. The first domain is a polymerase domain. The second domain is a nucleic acid binding domain that improves the processivity of the polymerase domain. Processivity is the ability of the polymerase to remain attached to the template and incorporate nucleotides into a second strand of nucleic acid that is being synthesized from the template.

The polymerase domain is defined by its function. The nucleic acid binding domain is defined by its percent identity to a prototype protein, Sso7d or Sac7d, and its ability to increase processivity of a polymerase to which it is joined. The claims argued specifically in this appeal are drawn to various embodiments in which the sequence non-specific double-stranded nucleic acid binding domain comprises at least 75% identity to the Sso7d reference sequence (claim 15); at least 75% identity to the Sac7d reference sequence (claim 30), at least 85% identity to the Sso7d reference sequence (claim 43), at least 85% identity to the Sac7d reference sequence (claim 44) and at least 90% identity to the Sac7d references sequence (claim 34).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The rejection of Claims 15, 17, 22-30, 32, and 34-44 under 35 U.S.C. § 112, first paragraph as not enabled is to be reviewed on appeal.

VII. ARGUMENT

A. Rejection and Examiner's Arguments

1. General summary of rejection

There is one rejection in the Final Office Action dated April 7, 2008. Claims 15, 17, 22-30, 32, and 34-44 are rejected under 35 U.S.C. § 112 for alleged lack of enablement. The Examiner's position is that it would require undue experimentation to determine an Sso7d and/or Sac7d variant that has at least 75%-90% identity to the reference Sso7d or Sac7d sequence, and that retains DNA binding activity and the ability to enhance processivity of a polymerase to which it is joined. The Examiner cites three references, which are discussed in detail below, as specifically teaching that sequence similarity alone does not necessarily provide a predictable correlation between the structure and specific function of a protein. The Examiner contends that neither the art nor the specification teach what "other" domains, regions or specific amino acids of Sso7d or Sac7d are responsible for sequence non-specific double-stranded DNA binding or enhancing processivity of an attached polymerase.

The Examiner acknowledges that the art, including the references that he cites, provides evidence of an association between double-stranded DNA binding activity and the ability to increase the processivity of an associated polymerase polypeptide. However, the Examiner maintains that this guidance is not specific beyond the fact that this relationship exists (see, *e.g.,* page 6, first sentence of the April 7, 2008 Final Office). The Examiner thus also disputes that the DNA binding activity of Sso7d is sufficiently connected to the ability to enhance processivity such that one of skill can recognize variants that would reasonably be expected to enhance processivity based on identification of residues involved in DNA binding in the Sso7d and Sac7d structures that are in the prior art.

2. Summary of rejection as it relates to three references cited by the Examiner in alleged support of the enablement rejection

The Examiner cites three references as evidence that allegedly support the rejection. These references are first cited in the Office Action mailed January 4, 2007. The

Examiner characterizes each of the references as teaching that a single point mutation of Sso7d affects the function of the nucleic acid binding domain and therefore demonstrating the unpredictability of determining variants that would have the claimed function. Specifically, a post-filing reference, Wang *et al. Nucl. Acids Res.* 32:1197-1207, 2004 ('Wang") co-authored by the inventor, is characterized by the Examiner as teaching that mutation of Trp24 of Sso7d significantly reduces its effectiveness in enhancing processivity. Wang is also cited as allegedly teaching that the use of a DNA binding protein with a much higher affinity for double-stranded DNA could be detrimental to the catalytic activity of the polymerase and that further studies are needed to identify the optimal range of affinities to achieve "the ultimate balance between processivity and catalysis" (Final Office Action, page 5). The Examiner describes Shehi *et al., Biochemistry* 42:8362-8368, 2003 ("Shehi"), another post-filing reference, as teaching that an Sso7d protein in which Glu53 is deleted could not be isolated and as suggesting that the mutation misfolds the protein. The Examiner also characterizes Shehi as teaching that an Sso7d protein having a deletion of Leu54 has limited solubility in aqueous solution. Last, Consonni *et al., Biochemistry* 38:12709-12717, 1999 ("Consonni"), is characterized by the Examiner as teaching that mutation of F31A and W23A in Sso7d impairs the capacity of the protein to bind dsDNA. The Examiner alleges that these various mutations demonstrate the unpredictability of the effect of point mutations in Sso7d on any particular function or attribute of Sso7d.

These references will be individually addressed in section VII.C.2.b.3.

B. Legal Standards for Enablement

It is well-settled in the biotechnology art that routine screening of even large numbers of samples is not undue experimentation when a probability of success exists. *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). As stated in *Wands*, "enablement is not precluded by the necessity for some experimentation, such as routine screening." *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988). The fact that experimentation may be complex does not render it undue.

As set forth by the Federal Circuit in *Wands* (*supra,* 8 USPQ2d 1400, 1404) multiple factors should be considered when determining whether any necessary experimentation is undue. These factors include:

(a) the breadth of the claims;

(b) the nature of the invention;

(c) the state of the prior art;

(d) the level of one of ordinary skill;

(e) the level of predictability in the art;

(f) the amount of direction provided by the inventor;

(g) the existence of working examples; and

(h) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

C. Claims 15, 17, 22-30, 32, and 34-44 are enabled.

The specification provides examples that show that both Sso7d and Sac7d increase processivity when joined to polymerases (*see, e.g.*, Figures 1 and 2), and directs the practitioner to the large body of art in this field that provides detailed structural insight into the interaction of Sso7d and Sac7d with DNA. In addition, a Declaration under 37 C.F.R. § 1.132 by Dr. Peter Vander Horn (Evidence Appendix A, submitted with Applicants' response filed March 2, 2004 and referred to herein as "the Vander Horn Declaration") provides objective

reasons, based on the detailed knowledge of Sso7d and Sac7d in the art, justifying the percent identities recited in the current claims.

Further, the prior art provides evidence that one of skill can in fact reasonably predict the effects of point mutations in Sso7d on double-stranded DNA binding and hence, on the ability of a variant protein to enhance processivity.

1. Teachings and examples in the specification

The specification teaches that the Archaeal small basic DNA binding proteins Sso7d and Sac7d and variants thereof having the recited percent identities can be used as DNA binding domains to enhance polymerase processivity when joined to polymerases. In particular, the specification provides reference sequences for the two proteins (SEQ ID NO:2 contains the Sso7d sequence, and SEQ ID NO:10 contains the Sac7d sequence), which were characterized in the art prior to Applicants' invention, and directs a practitioner to exemplary references describing such studies (*e.g.*, Baumann *et al. Structural Biol* 1:808-819, 1994 and Gao *et al., Nature Struc. Biol* 5:782-786, 1998; both cited at page 12, lines 8-15; copies provided as Exhibits 9 and 3, respectively, of the Vander Horn Declaration). In addition, the application provides general guidance for determining percent identity using well known methods (*see, e.g.,* the section beginning on page 14, line 5 of the specification) and for performing functional assays. The functional assays include those that evaluate sequence non-specific double-stranded DNA binding activity of a DNA binding domain, and assay to evaluate modified polymerases for enhanced processivity (*see, e.g.,* page 28, lines 16-33).

Furthermore, the specification exemplifies both Sso7d and Sac7d polymerase fusion proteins. First, the specification provides data showing that that Sso7d enhances processivity of both *Taq* and *Pfu* polymerases (*see, e.g.,* page 34). The two polymerase are in different polymerase families. *Taq* is a family A polymerase; *Pfu* is a family B polymerase (see, *e.g.,* Example I, first paragraph on page 31 and page 32, second full paragraph). The specification additionally provides data demonstrating that that Sso7d can be joined at either its N-terminus or C-terminus to the polymerase (*see, e.g.,* the description of the construction of

fusion polymerases that begins on page 32). In Sso7d-*Taq* fusions, Sso7d is joined through its C-terminus to the N-terminus of *Taq* or Δ*Taq*. In the *Pfu*-Sso7d fusion, Sso7d is joined through its N-terminus to the C-terminus of *Pfu* polymerase. These examples thus show that Sso7d, modified at either the N-terminus or C-terminus by linkage to the polymerase, can increase the processivity of polymerases.

The specification also provides data demonstrating that Sac7d, which has 82% identity to the Sso7d reference sequence SEQ ID NO:2 (*see, e.g.,* the Vander Horn Declaration at section 12 beginning on page 7) has the same effect on a polymerase as that observed with Sso7d. In Example 4 at page 36, lines 27-30, a Sac7d-ΔTaq fusion was evaluated in a PCR reaction using short primers. The results (Figure 2) show that the Sac7d polymerase fusion was very similar to the Sso7d polymerase fusion.

The specification thus provides ample teachings, including working examples, to guide one of ordinary skill in the art in practicing the claimed invention.

2. State of the art at the time of the invention

The Sso7d and Sac7d prototype sequences are not novel genes. Applicants are not claiming 75% identity to arecently discovered prototype gene. There is an extensive body of literature in the art pertaining to the structure of Sso7d and Sac7d. In the Vander Horn Declaration, Dr. Vander Horn explains that Sso7d and Sac7d are part of a family of naturally occurring Archaeal proteins (referred to herein for convenience as "Sso7" proteins). A natural variation of about 76% occurs within the family (*see, e.g.,* section 7 of the Vander Horn Declaration, beginning on page 2, which is discussed at greater length below). Further, analyses of the structures of Sso7d and Sac7d bound to DNA have been performed by several investigators. Dr. Vander Horn illustrates how this structural information is used to select amino acid residues for substitution that can reasonably be expected to preserve DNA binding function and accordingly, the ability to influence polymerase processivity (*e.g.*, section 10 of the Vander Horn Declaration beginning on page 4, as explained below).

a. Applicants have provided objective reasons justifying the percent identities set forth in the claims based on known sequences.

Not only does the subject specification provide a full disclosure of the family of Sso7 proteins, Applicants have provided the Vander Horn declaration, which provides objective reasons justifying the 75% identity level. Dr. Vander Horn explains that by routinely comparing the sequence differences between the family members, those of skill would immediately recognize where the critical and noncritical regions of the proteins are located. The family members are a virtual roadmap to novel variants. Dr. Vander Horn additionally explains how the prior art, *e.g.*, Gao *et al.*, provide structure-activity relationships that can be used in determining residues that can reasonably be expected to be substituted without compromising activity.

According to Dr. Vander Horn, a GenBank search of Sso7d readily identifies 17 naturally occurring DNA binding proteins that have amino acid identities of between 98-79% (*e.g.*, section 7 of the Vander Horn Declaration). Indeed, in section 12 of his declaration, Dr. Vander Horn explains that based on naturally occurring proteins alone, domains having 79% identity to Sso7d or Sac7d are readily available for use in the invention. The second paragraph of page 18 of the Declaration further notes that three of the references cited in the specification (Choli *et al.*, Baumann *et al.*, and McAfee *et al.*, copies of which are provided as exhibits to the Vander Horn Declaration) contain figures with sequence alignments of Sso7d homologues, including Sac7d, Sac7a and Sac7e. These proteins are repeatedly described as structurally and functionally closely related proteins. Dr. Vander Horn concludes that "[n]o one skilled in the arts that reads the patent specification and the referenced papers would have objective reasons to think it [the proteins] wouldn't work."

In section 13 of his Declaration, Dr. Vander Horn illustrates how one of skill can readily generate a protein having 76% identity to Sso7d using the natural variation that occurs in Sso7 family members as a road map. In addition to the natural variations between family members, one of skill in the art readily understands that non-naturally occurring but conserved substitutions are possible throughout the primary sequences of the prototype proteins. Dr. Vander Horn explains this conventional wisdom at section 9 of his Declaration.

Dr. Vander Horn further notes that man-made modifications (muteins) can additionally be generated by introducing conservative substitutions at sites selected based on structural information (discussed below). Such a procedure can readily generate a protein having lower than 60% identity to the reference Sso7d sequence that still would enhance polymerase processivity (section 14, beginning on page 8).

b. Applicants have provided objective reasons justifying the percent identity set forth in the claims based on structure of the protein.

Dr. Vander Horn explains at section 10, beginning on page 4 that the structure of Archaeal proteins when complexed with DNA has been previously studied by investigators such as Gao *et al.* Dr Vander Horn details how this information permits a practitioner to identify the critical DNA binding regions in the proteins, which allows one of skill to focus mutations away from these critical regions. Specifically, Dr. Vander Horn points to unstructured regions of Sso7d (first full paragraph on page 5), which are sites where divergences in Sso7 sequences occur, that can be targeted for mutations. Dr. Vander Horn also indicates that residues in the alpha helix, which do not interact with the DNA substrate, could be targeted for substitution so long as they preserved secondary structure (second paragraph of page 5). Furthermore, based on the structures, Dr. Vander Horn explains that the differences in composition and length between Sso7 and Sac7 cluster in the turns between beta sheets and in amino acids facing away from the DNA binding domain in the crystal structure and that these regions are thus also areas of plasticity. Finally, various lysine residues that would be reasonably be expected to tolerate substitution without compromise to DNA binding are described in paragraphs 4 and 5 on page 5.

The Vander Horn Declaration thus illustrates how one of skill in the art can use the large body of knowledge in the art to identify functional Sso7d and Sac7d variants having the percent identity set forth in the claims without undue experimentation. Therefore, in view of the guidance provided in the specification, the existence of working examples, the level of skill of the ordinary practitioner in the art, and the depth of knowledge in this art, the claims are properly enabled over the entire scope.

3. The predictably in the art

The predictability in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. What is known in the art provides evidence as to the question of predictability. MPEP §2164.03. In the present case, the basic techniques used in practicing the invention, *e.g.,* those used in the art of recombinant expression and protein analysis, have been in existence for over two decades and have since improved dramatically to reach a high level of technical sophistication and predictability. In addition, as discussed above, much is known about the structural and functional features of Sso7d and Sac7d that allow one of skill to rationally predict the effects of changes in protein sequence. Therefore, a significant level of predictability exists in the relevant art.

The Examiner cited three references in support of the position that it would require undue experimentation to make and use Sso7d and Sac7d variants in the invention. The references are characterized by the Examiner as showing that a single point mutation in Ssod can affect the function of the nucleic acid binding domain and therefore demonstrating that the effects of mutating Sso7d can not be reasonably expected to be predictable by one of ordinary skill in this art. However, the references in fact support the enablement of the claims. Applicants have submitted a Declaration under 37 C.F.R. § 1.132 by Dr. Yan Wang (the Wang Declaration) that is provided in the accompanying appendix, which explains that the experiments performed in the cited publications provide evidence that shows that one of skill can reasonably be expected to be able to use the extensive structural Sso7d/Sac7d data available in the art to predict the effects of sequence changes on Sso7d (or Sac7d) DNA binding activity. The Wang Declaration also explains that the cited art additionally shows that DNA binding activity correlates with the ability to modulate processivity of a polymerase to which the Sso7d/Sac7d protein is joined.

In the cited references the authors were seeking to investigate Sso7d by introducing mutations that were predicted, based on the structure, to negatively affect function. Dr. Wang illustrates how their results validate this approach of using the structure to predict effects on function. In the current invention, the skilled artisan can use this same structural

information to reasonably predict sequence changes that preserve Sso7 function, rather than destroy it. Each of the references is individually discussed below.

Wang

The Examiner points to Wang as further supporting the rejection because Wang teaches that a change in Trp24 of Sso7d significantly reduces the effectiveness of the protein in enhancing processivity. Wang is a post-filing publication of the current inventor's work relating to polymerases that are modified by linkage to an Sso7d protein. In the Wang Declaration, Dr. Wang explains that in one aspect of the experiments presented in the article, it was determined that Sso7d double-stranded DNA (dsDNA) binding activity is important for processivity, as taught in the current application. As Applicant has previously noted, the interactions between Sso7d and dsDNA have been extensively studied. Dr. Wang explains that for the experiments described in her publication, the Wang reference, Trp 24 was identified in structural studies to be important for binding to dsDNA, as explained on page 1201, column 1 in the last paragraph. (Trp24 in Wang corresponds to Trp23 in SEQ ID NO:2 of the application as filed.) The referenced structural studies (Gao *et al., Nature Struct. Biol.* 5:782-786, 1998; and Catanzano, *et al. Biochemistry* 37:10493-10498, 1998) were readily available in the art before the current invention. In the Declaration, Dr. Wang further explains that she purposefully selected Trp 24 for mutation in the studies described in the Wang publication to further investigate the correlation between DNA binding and processivity. Three mutant Sso7d-polymerase fusion proteins in which Trp 24 was replaced with Val, Gly or Glu were created with the intent of reducing the ability of Sso7d to bind dsDNA and in turn, reducing its ability to enhance the processivity of the DNA polymerase. Dr. Wang points out that all three mutant fusion proteins exhibited decreased processivity relative to that of the wildtype Sso7d-polymerase fusion, just as they had expected. Substitution of Trp 24 with Glu, which had been expected to exhibit the greatest effect because it differs the most from the wild-type residue, also resulted in the greatest decrease in processivity.

The experiments presented in the Wang publication therefore illustrate how one of skill in the art makes use of available structural information to recognize amino acid residues that

are expected to be relevant to function. Wang and her co-authors intentionally selected a residue that is integral to DNA binding activity based on available Sso7d structural data, fully expecting to compromise the function of Sso7d in enhancing polymerase processivity. This is precisely what was observed. Therefore, one of skill can in fact make predictions based on structural information that have the desired effect on function. The same structural information can be used to select residues that would not be expected to alter Sso7d activity.

Consonni

Consonni is cited by the Examiner as providing evidence that the claims are not enabled because a single amino acid change (at Trp 23 or Phe 31) in Sso7d can alter function. However, Consonni also provides another example of how structural information is predictive of the functional importance of particular amino acid residues. In the Wang Declaration, Dr. Wang explains that Consonni describes the solution structure of an Sso7d mutant protein F31A, in which an alanine is substituted for a phenylalanine residue at position 31. In prior studies cited in Consonni at page 12710 in the second full paragraph of the first column, Phe 31 was selected for mutation on the basis of structural data that indicated that this residue is located at the core of the aromatic cluster and has tight contact with side chains of several residues in the cluster. This residue was therefore predicted to be important for Sso7d stability.

Dr. Wang further notes that this residue is also highly conserved in Sso7 family members, as can be seen in a sequence comparison of Sso7d, Sac7d, Sac7a, and Sac7e (see, the Vander Horn Declaration). As the authors expected, the mutation of Phe 31 to Ala led to a loss in thermo and piezostabilities (third paragraph of column 1, page 12710). The analysis presented in the Consonni paper cited by the Examiner relates to the solution structure of the F31A mutation, which was performed in order to determine the structural changes that were associated with the loss of stability of the mutant protein.

Dr. Wang points out that Consonni observed that in the solution structure of the F31A mutant, the Trp 23 residue was reoriented such that it pointed inside the aromatic cluster. Because of the previously identified role of Trp23 in contacting DNA (Trp 23 is the same residue

as Trp24 in Wang), Consonni investigated the DNA-binding activity of the mutant F31A protein. The results showed that the binding activity was also impaired, once more highlighting that Trp 23 plays an important role in DNA binding, as indicated by the structure.

With regard to the loss of stability observed in the F31A mutant protein, Dr. Wang indicates that it is not surprising that the mutation affected Sso7d stability. As explained in the Wang Declaration, it is well known in the field that an amino acid with a large, buried hydrophobic side chain stabilizes conformation. Accordingly, it is predictable that changing the large hydrophobic side chain to a small side chain would result in a loss of stability. In designing mutations that are expected to preserve function, Dr. Wang further notes that it is standard practice in the art to avoid radically mutating such residues, if it is desired to preserve function, just as it would be desirable to avoid mutating those residues that directly contact DNA to preserve DNA binding function.

Shehi

Shehi investigated the function of the C-terminus of Sso7d. Shehi created an Sso7d protein that was truncated at Leu54 (L54Δ) in order to investigate the role of the C-terminal α-helix on stability and DNA binding activity. Dr. Wang notes that this region does not contact the DNA in the structural studies of Sso7d and Sac7d DNA binding interactions. Dr. Wang then explains that to determine whether deletion of the C-terminal region had effected DNA binding, the authors analyzed the binding of L54Δ to double-stranded calf thymus DNA in comparison to the binding activity of wildtype Sso7d. Dr. Wang points out in her Declaration that the association constant for binding of L54Δ to double stranded DNA was similar to that of Sso7d, thus showing that deletion of the eight residues at the C-terminus of Sso7d did not result in loss of DNA binding activity, which was predictable based on the structure.

The authors also observed that a variant that was truncated at Glu 53 could not be isolated under the same conditions that allowed them to isolate L54Δ and noted that this highlights the role that Leu 54 plays in the folding process. Shehi observes that Baumann and colleagues (*Nat. Struc. Biol.* 1:808--809, 1994) in fact described that the side chain of Leu54 is

packed well against that of Ala50, anchoring the C-terminal end of the chain to the protein core. Other investigators also confirmed that Leu54 is involved in strong van der Waals interactions with the remaining part of the protein. Thus, as Dr. Wang indicates, the available Sso7d/Sac7d structural data provided information on the role of Leu 54 that was borne out by Shehi's studies.

Dr. Wang further explains that Shehi's results are consistent with the analysis of Sso7d structure provided by Vander Horn Declaration that is of record in this application. Dr. Wang points out that Dr. Vander Horn has indicated that in the context of DNA binding activity, the alpha helix is highly mutable, as evidenced by the fact that natural variation of Sso7 homologs is observed in this domain. Dr. Vander Horn cautioned, however, that the naturally occurring mutations in this domain appear to preserve the alpha helix. Thus, in designing Sso7d variants for use in the invention, one of skill would introduce mutations that preserved structure. Dr. Wang further notes that the L54 residue is also conserved across the naturally occurring Sso7 proteins, which also would be an additional consideration in designing variants.

Shehi mentions that there were difficulties in isolating the deletion in which the C-terminus was truncated at Glu53 under the same conditions that were used to isolate L54Δ. Shehi also noted that L54Δ has a limited solubility in aqueous solution. The Examiner contends that "both mutations demonstrate the unpredictability of the effect of point mutations in Sso7d on any particular function or attribute of Sso7d." However, Dr. Wang explains that one of skill cannot conclude from the experiments in Shehi that the effects of point mutations at Glu53 or L54 would be unpredictable. Dr. Wang first notes that Shehi investigated deletion mutations, not point mutations, and that the effects observed in deleting most of the C-terminal α-helix cannot be extrapolated to the effects of introducing point mutations into that region.

In terms of the limited solubility of L54Δ, the authors believe that this is likely due to the loss of three net charges and the exposure of hydrophobic moieties upon deleting the last eight residues. Dr. Wang points out that it is recognized in the art that changing the charge of a protein and exposing hydrophobic residues can influence solubility. She indicates that a practitioner in this art can additionally consider such effects in designing variant Sso7d sequences. Last, she notes that Shehi was examining L54Δ alone, not when fused to a

polymerase protein. This is relevant because, the limited solubility observed by Shehi under these conditions would not necessarily reflect the solubility when the protein is fused to a polymerase. In view of the foregoing, the studies in Shehi do not provide evidence that the current claims are not properly enabled.

As illustrated in the Wang Declaration the three references cited by the Examiner demonstrate that prior art structural information about Sso7d/Sac7d provides a sound basis for rationally predicting of effects of mutations in Sso7d and Sac7d on DNA binding function and the ability of the protein to increase processivity of a polymerase to which it is joined.

4. Quantity of experimentation

Appellants do not dispute that some experimentation may be necessary to practice the present invention as defined by the pending claims. Yet "the test [of undue experimentation] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt and Griffen*, 190 USPQ 214, 217-19, (CCPA 1976)).

As MPEP §2164.01 states, complex experimentation is not necessarily undue, if the art typically engages in such experimentation. Because any necessary experimentation for practicing the claimed invention in the instant case would be routine for an ordinarily skilled artisan who is familiar with the well established techniques of protein analysis and molecular biology, such experimentation does not constitute undue experimentation.

In order to enable a generic claim, Applicants need not enable every conceivable species, but only provide guidance sufficient that that one of skill could reasonably expect that mutations could be introduced that would have predictable effects (*In re Angstadt* 190 USPQ 214 (CCPA 1976). The art cited by the Examiner provides additional evidence that one of skill could reasonably expect that the structural information available for Sso7d and Sac7d can be

used as a basis for reasonably predicting whether a substitution would affect DNA binding and processivity.

The Examiner argues that there is insufficient in-depth explanation in the specification as to exactly how DNA binding and processivity effects are related. Wang is also cited in the April 7, 2008 final Office Action as teaching that the use of a DNA binding protein with a much higher affinity for double-stranded DNA could be detrimental to the catalytic activity of the polymerase and that further studies are needed to identify the optimal range of affinities to achieve "the ultimate balance between processivity and catalysis". However, the claims do not require that the polymerases have the ultimate balance between processivity and catalysis. Optimization is not a requirement for patentability. The evidence as a whole suggests that DNA binding activity and ability to enhance polymerase processivity are sufficiently connected such that the prior art structures of Sso7d and Sac7d complexed to DNA allow one of skill to reasonably predict amino acid residues that can be substituted without comprising the ability to increase processivity. One of skill can therefore practice the invention without undue experimentation.

D. Claims 43 and 44 are additionally enabled.

Claims 43 and 44 are also argued independently.

Claims 43 and 44 relate to a modified polymerase that has a sequence-non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 85% identity to SEQ ID NO:2 or to the Sac7d sequence of SEQ ID NO:10. Claims 43 and 44 are enabled for the reasons explained above and for additional reasons. As noted above, the examples in the specification show that both Sac7d and Sso7d work in the claimed invention. These two proteins, relative to one another, are two of the most divergent members of the naturally occurring family members (*see, e.g.,* section 7 of the Vander Horn Declaration). If claims reciting at least 75% identity to the reference sequence, which encompasses all 18 of the naturally occurring Sso7d and Sac7d-related proteins identified by Dr. Vander Horn in his search, are not deemed to be enabled by the specification despite the facts detailed above, then it

is submitted that claims directed to at least 85% identity should be allowable. Such proteins would be more closely related than the most divergent members. For example, with Sso7d there are 12 residues of the 63 residues in which natural variation are known. The limit of 85% identity would encompass variants that have less than the full range of variation, but still allow most changes that could be introduced into an Sso7d sequence based on the naturally occurring variation. For the reasons explained in the Vander Horn Declaration, such changes would reasonably be expected to retain function, as the naturally occurring family members have the same function. The same reasoning applies to proteins having at least 85% identity to Sac7d. Accordingly, claims drawn to protein domains having at least 85% identity to Sso7d or Sac7d are additionally enabled.

E. Claim 34 is additionally enabled.

Claim 34 is additionally argued independently.

Claim 34 relates to a modified polymerase that has a sequence-non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 90% sequence identity to the Sac 7d sequence set forth in SEQ ID NO:10. Claim 34 is enabled for all of the reasons explained above and for additional reasons. Sac7d variants having at least 90% identity to the reference sequence are largely unchanged in protein sequence relative to the reference sequence. In view of the knowledge in the art and as evidenced by the Vander Horn Declaration, one of skill could reasonably be expected to generate variants having such minor changes that would be expected to retain DNA binding activity and hence, the ability to enhance processivity. Furthermore, claims relating to Sso7d-polymerases in which the Sso7d domain has at least 90% identity to the reference Sso7d sequence were deemed patentable by the Patent Office (see, parent application, now U.S. Patent No. 6,627,424.) The same facts supporting the patentability of those claims would logically apply to claim 34 of the current application.

F. Legal precedent supports the allowing claims of the scope presently pending.

Beyond the objective evidence provided above, legal precedent supports the Examiner allowing claims of the scope presently pending. In the current invention, Applicant is fusing two known protein families. The inventive principle is improving the processivity of polymerases by fusing them with an Archaeal DNA binding domain. The inventive principle is not a polymerase, nor is it an Archaeal DNA binding protein. There is a body of case law that focuses on the importance of inventive principle in considering adequacy of support in the specification for broad claims. Three cases are particularly illustrative.

In *In re Fuetterer*, 319 F.2d 259, 138 USPQ 217 (CCPA 1963), the applicant had discovered that the addition of a protein with an "inorganic salt" to the materials used to make tire tread increased the stopping ability of tires made from the materials. The examiner argued that the recitation of "inorganic salts" rendered the claims too broad because the amount of experimentation required to successfully use undisclosed inorganic salts was undue and required the application to restrict the claims to the disclosed salts. The CCPA reversed the breadth rejection, explaining that this invention was the combination of inorganic salts with the other elements of the claims. The fact that novel inorganic salts might be later developed did not preclude broad claims to the inventive combination.

Application of Herschler, 200 USPQ 711 (CCPA 1979) is additionally instructive in clarifying enablement requirements regarding claims reciting old elements Although the decision in this case is in the context of written description, the same analysis with respect to the issue of inventive principle applies to the enablement rejection raised by the Examiner against the present claims.

In *Herschler*, the applicant had discovered that dimethylsulfoxide (DMSO) was useful as a transdermal carrier for physiologically active steroids. The CCPA found that a priority application describing a single steroid (dexamethasone 21-phosphate) supported a claim to the genus of all steroids. The CCPA explained that Herschler's claims were not drawn to a novel steroid but to a method of administering steroids. As long as the class of steroids could be

expected to be carried across the skin by DMSO, the claim could encompass any steroid, known or unknown. Following earlier case law, the CCPA reminded the Patent Office that the "inventive principle" was directed to a method of administration of steroids and that the specific steroid exemplified was not the point of patentability.

Herschler provides guidance in identifying the inventive principle and its effect on questions of written description and enablement. There the court stated:

> The solicitor urges that the class of steroids is so large that a single example in the specification could not describe the varied members with their further varied properties. We disagree with this contention. Steroids, when considered as drugs, have a broad scope of physiological activity. On the other hand, steroids, when considered as a class of compounds carried through a layer of skin by DMSO, appear on this record to be chemically quite similar. (*Herschler*, at 717)

The CCPA is saying that the PTO mistakenly focused its concern on the claim element "steroids." Logically following that error, the PTO then argued that all steroids were not yet known and therefore any claim embracing the entire genus was not properly supported. This was an irrelevant truth because the initial premise was in error: the inventive element was not steroids; but their use in combination with a transdermal carrier.

In re Lange, 209 USPQ 288 (CCPA 1981) further emphasizes the importance of inventive principle. In *Lange*, the invention related to a circuit breaker that quenches an electric arc produced between electrodes by use of an electronegative gas. The PTO argued that the application only taught how to coat electrodes with the gases and not how to forge them with the gases. This was true, but the court recognized that the invention was not how to make electrodes but the discovery that the use of the gases would prevent arcing ("the method of forming the electrodes is not the inventive principle."*Lange*, at p. 295). The court further stated that:

> Although appellant can be required to limit his claims to that subject area that is adequately disclosed, existence of species that are not adequately disclosed does not require that entire application be found nonenabling; this is especially true in case in which inadequately disclosed method is not inventive principle. (*Lange*, at 289).

Thus, the inventive principle in *Fuetterer* was the "use" of inorganic salts with the other elements of the claims; in *Herschler* it was the "use" of DMSO to transdermally transport

all steroids; and in *Lange* the inventive principle was the "use" of gases to prevent arcing. In a parallel fashion, the instant invention concerns the "use" of an Archaeal sequence non-specific double-stranded nucleic acid binding protein to improve processivity of a polymerase.

The Examiner makes much argument about the failure of the specification to discuss all Archaeal Sso7d proteins. The fact that not all Archaeal binding proteins are known is an irrelevant truth because that degree of enablement is not required to allow a claim that does not rely on that element for its patentability. One of skill would understand that many DNA binding proteins from Archaeons, as a genus, are capable of binding double-stranded DNA nonspecifically. And, if provided with a novel protein, one of skill could easily determine, with no undue experimentation, whether or not the novel protein binds nonspecifically to nucleic acid.

The case law cited by the Examiner does not properly support the Examiner's position.

The Examiner relied on *In re Fisher*, 166 USPQ18 (CCPA 1970) to support his position. *Fisher*, however, is not applicable to the facts presented here. In *Fisher*, the invention was a hormone, ACTH, that has 39 amino acids. The inventors determined that the first 24 residues of ACTH are conserved across several animals. The rejected claims read on any ACTH protein in which the first 24 amino acid residues were the conserved sequence and that sequence was specifically recited in the claim. While such a claim may not be a problematic claim today, in 1970 it was not technically possible to make ACTH chemically and all the natural known species had 39 amino acids. Because there was no way to make an ACTH of other than 39 amino acids in length, the claim was properly rejected by the CCPA as non-enabled. As the court said:

> We have already discussed, with respect to the parent application, the lack of teaching of how to obtain other-than-39 amino acid ACTHs. That discussion is fully applicable to the instant application, and we think the board was correct in finding insufficient disclosure due to this broad aspect of the claims (*In re Fisher*, at 23)

Procedurally, the rejection of a claim to a protein reciting a "signature sequence" is no longer an issue because of advances in protein chemistry. There was nothing inherently wrong with the

Fisher claim structure—it was simply written before technology could enable it. That is not true in our situation. Following natural variations as a road map and applying routine mutagenesis techniques, those of skill can routinely create variations of Sso7d and Sac7d that are at least 75% identical to each other or greater.

Recent Board decision supports allowing the claims.

Appellants request that the Patent Office take note of the Board's recent decision in *Ex parte Yuejin Sun et. al.* (unpublished decision, Appeal No. 2003-1993, Bd. Pat. App. Int., Jan. 20, 2004). Although the *Sun* decision was unpublished, the facts are so similar to Applicants' circumstances that the opinion is powerfully persuasive in favor of allowing the pending claims. In *Sun*, the invention was a novel plant gene encoding a protein called 'wee1'. The claims at issue claimed a nucleic acid having "at least 80% identity to the entire coding region of SEQ ID No: 1." The examiner had applied both a description and enablement rejection. The Board of Appeals reversed both the description and enablement rejections.

To support the enablement rejection, the examiner in *Sun* employed the same arguments presented in the Final Office Action. Those arguments were: (i) there was no structure activity relationship; (ii) there were no predictable means taught for modifying the prototype coding region to 80% identity while retaining activity; and, (iii) there were insufficient examples. Although not cited by name, the Board reversed the rejections applying the principle set forth in *In re Angstadt and Griffen*, 190 USPQ 214 (CCPA 1976). In *Angstad*, the CCPA ruled that claims that embraced some non-working embodiments were permitted under §112 so long as a functional assay was provided that allowed those of skill to routinely avoid non-working embodiments. In *Sun*, the Board recognized that the appealed claim was enabled by the disclosure of a functional assay to routinely determine when you had proteins that functioned and by the fact that modifications to the primary amino acid sequence of wee 1 were also routine.

In comparison to *Sun*, the facts of the instant case are even more compelling towards claim allowance. In *Sun*, the gene was novel and was the invention *per se*. In the

instant application, the recited gene family is a claim element that is both well known and well characterized. Applicants have provided objective evidence that the claim limitation of 75% to 85% identity to Sso7d and 75% to 90% identity to Sac7d is a reasonable approximation of the ability of protein chemists to alter the primary sequence of the prototype while maintaining biological function.

Further, the facts in *Sun* can be distinguished from another recent Board Decision *Ex parte Cortese et al.* (unpublished decision, Appeal No. 2008-0763, Bd. Pat. App. Int., Jan. 24, 2008). In *Cortese*, the claims at issue related to methods of screening for compounds that inhibit SR-B1 activity by using an assay that employs an SR-B1 polypeptide that is at least 95% similar to a human SR-BI reference sequence and an HCV E2 polypeptide to which the SR-B1 polypeptide binds. The Board upheld the Examiner's rejection of the claims as lacking proper written description and as not enabled.

Unlike *Sun*, the specification did not identify the regions or regions in the 509 amino acid SR-B1 protein that are involved in HCV E2 binding. Nor did the prior art. Furthermore, a mouse SR-BI homolog that had 80% homology to the human sequence did not have the HCV E2 binding activity. The Board found that the genus of SR-B1 proteins that are at least 95% similar to SR-BI and that bind to HCV E2 was not properly described in the absence of knowledge of which structural elements of SR-BI are involved in HCV E2 binding.

The Board also affirmed the enablement rejection. Although the Board again noted that there was no description of the HCV E2 binding region of SR-BI in upholding the rejection, the Board's decision largely focused on the breadth of the SR-BI activities encompassed by the claims. The Board noted that the claims encompassed inhibition of activities of SR-BI variants other than the HCV E2 binding activity. The Board found that the prior art cited by the Examiner demonstrated that compounds that inhibit one SR-BI activity would not necessarily have any effect on any other SR-BI activity. The Board described this as the essence of unpredictability and affirmed the rejection for lack of enablement.

Here, the facts are different. The specification provides working examples and points the practitioner to Sso7d/Sac7d structure data that teach residues involved in DNA

binding. Further, as explained in the Vander Horn Declaration one of skill can reasonably predict functional effects from the protein structure. This is borne out by the studies described in the three references cited by the Examiner. Last, there is a sufficient correlation between double-stranded DNA binding activity and the ability to increase the processivity of a polymerase such that one of skill can reasonably predict which Sso7d and Sac7d variant proteins will enhance processivity based on their DNA binding activity.

Conclusion

Policy Considerations

For the reason explained above, the claims are compliant with the standards for enablement. It is respectfully requested that the outstanding rejection be reversed.

During prosecution, the claims in the context of the decision in *Ex parte Sun* were discussed with the Examiner and Supervising Examiner. The decision in *Sun* related to a novel protein and 80% identity was considered to be enabled. Here, the claims are not drawn to a novel protein and neither 75%, 85%, nor 90% identity is considered by the Examiner to be enabled. Applicants respectfully request that the decision for this appeal be considered for possible publication in order to provide guidance and clarify patent office policy on protein claims that are cast in terms of percent identity.

Respectfully submitted,

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VIII. CLAIMS APPENDIX

1.-14. (cancelled)

15. (previously presented) A protein comprising two joined heterologous domains:

a sequence non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 75% sequence identity to SEQ ID NO:2; and

a DNA polymerase domain

wherein the presence of the sequence non-specific double-stranded nucleic acid binding domain enhances the processivity of the polymerase domain compared to an identical protein that does not have the sequence non-specific double-stranded nucleic acid binding domain joined thereto.

16. (cancelled)

17. (previously presented) The protein of claim 15, wherein the sequence non-specific double-stranded nucleic acid binding domain and the DNA polymerase domain are covalently linked.

18.-21. (cancelled).

22. (previously presented) The protein of claim 15, wherein the DNA polymerase domain has thermally stable polymerase activity.

23. (previously presented) The protein of claim 15, wherein the DNA polymerase domain comprises a family A polymerase domain.

24. (previously presented) The protein of claim 23, wherein the family A polymerase domain is a *Thermus* polymerase domain.

25. (previously presented) The protein of claim 23, wherein the family A polymerase domain polymerase domain is a *Taq* polymerase domain.

26. (previously presented) The protein of claim 22, wherein the DNA polymerase domain is a Δ*Taq* domain.

27. (previously presented) The protein of claim 15, wherein the polymerase domain is a family B polymerase domain.

28. (previously presented) The protein of claim 27, wherein the family B polymerase domain is a *Pyrococcus* DNA polymerase I domain.

29. (previously presented) The protein of claim 28, wherein the *Pyrococcus* polymerase domain is a *Pyrococcus furiosus* domain.

30. (previously presented) A protein comprising two joined heterologous domains:

a sequence non-specific double-stranded nucleic acid binding domain that comprises an amino acid sequence that has at least 75% sequence identity to the Sac7d sequence set forth in amino acids 7-71 of SEQ ID NO:10; and

a DNA polymerase domain,

wherein the presence of the sequence non-specific double-stranded nucleic acid binding domain enhances the processivity of the polymerase domain compared to an identical protein that does not have the sequence non-specific double-stranded nucleic acid binding domain joined thereto.

31. (cancelled)

32. (previously presented) The protein of claim 30, wherein the sequence non-specific double-stranded nucleic acid binding domain and the DNA polymerase domain are covalently linked.

33. (cancelled)

34. (previously presented) The protein of claim 30, wherein the sequence non-specific double-stranded nucleic acid binding domain comprises an amino acid sequence that has at least 90% sequence identity to the Sac 7d sequence set forth in SEQ ID NO:10.

35. (previously presented) The protein of claim 30, wherein the DNA polymerase domain has thermally stable polymerase activity.

36. (previously presented) The protein of claim 30, wherein the DNA polymerase domain comprises a family A polymerase domain.

37. (previously presented) The protein of claim 35, wherein the DNA polymerase domain is a *Thermus* polymerase domain.

38. (previously presented) The protein of claim 36, wherein the *Thermus* polymerase domain polymerase domain is a *Taq* polymerase domain.

39. (previously presented) The protein of claim 35, wherein the DNA polymerase domain is a Δ*Taq* domain.

40. (previously presented) The protein of claim 30, wherein the polymerase domain is a family B polymerase domain.

41. (previously presented) The protein of claim 40, wherein the family B polymerase domain is a *Pyrococcus* DNA polymerase I domain.

42. (previously presented) The protein of claim 41, wherein the *Pyrococcus* polymerase domain is a *Pyrococcus furiosus* domain.

43. (previously presented) The protein of claim 15, wherein the sequence non-specific double-stranded nucleic acid binding domain comprises an amino acid sequence that has at least 85% sequence identity to SEQ ID NO:2.

44. (previously presented) The protein of claim 30, wherein the sequence non-specific double-stranded nucleic acid binding domain comprises an amino acid sequence that has at least 85% sequence identity to the Sac 7d sequence set forth in SEQ ID NO:10.

IX. EVIDENCE APPENDIX

Submitted with Appellants' Brief filed September 23, 2008.

X. RELATED PROCEEDINGS APPENDIX

None